



General Description

DNA pHAGE2-EF1aFull-hOct4-F2A-hKlf4-IRES-hSox2-P2A-hcMyc-W-loxP
Entire molecule length: 12535 bp

Standard Fields

User Fields

Author(s)

Author

Original author

A Omari

References

Comments

10/23/08 Aba Somers annealed oligos designed to ligate into pHAGE 2 3' LTR after backbone is cut ligation creates 5' Asc1 site and 3' BfuA1 site.
Introduces loxP sequence into 3'LTR, along with new Kpn1 site into backbone (use this for analytical

11/11//08 AS Clone 12 maxiprep: confirmed perfect insert sequence including Asc1 ligation junction.

Feature Map

CDS (7 total)

HIV gag

Start: 790 End: 1144

HIV gag sequence from 336bp to 688bp (Clone to PvuII site in HIV and filled in ClaI digest to

env

Start: 1156 End: 2013

HIV env sequence from bp7168 to 8025 (contains RRE) Cloned from BglII to BamHI out of HIV

hOct4

Start: 3412 End: 4491

human Klf4

Start: 4576 End: 5988

hSox2

Start: 6576 End: 7526

hcMyc

Start: 7596 End: 8915

AMPr

Start: 10149 End: 10988

Ampicillin Resistance Gene

Original Location Description:

6153..6992

Qualifiers:

/gene="AMPr"

/product="beta-lactamase (mature form)"

Intron (1 total)

Intron

Start: 2454 End: 3045

LTR (2 total)

5' LTR

Start: 1 End: 636

Long Terminal Repeat

Original Location Description:

1..636

Qualifiers:

/gene="LTR"

3' LTR

Start: 9524 End: 9893

Misc. Feature (28 total)

HIV U3

Start: 1 End: 454

Full Length HIV U3

HIV R

Start: 455 End: 551

HIV Repeat Region

HIV U5

Start: 552 End: 636

HIV U5

PBS-K

Start: 636 End: 658

tRNA binding site for Lysine tRNA

PSI

Start: 637 End: 1155

PSI Packaging Sequence

Original Location Description:

637..1155

Qualifiers:

/gene="psi"

/product="pbs-gag"

HIV-PSI

Start: 697 End: 806

HR-ePSI

Start: 807 End: 1144

HIV gag sequence 351bp to 688bp

Stop Codon

Start: 853 End: 855

Stop codon in gag put in frame by ClaI fill in

RRE

Start: 1245 End: 1593

Rev Responsive Element according to Belasco in Molecular Cell, Vol. 7, 603-614, March, 2001

RRE

Start: 1303 End: 1536

Rev Responsive Element

Original Location Description:

1303..1536

Qualifiers:

/gene="rre"

/product="minimal RRE"

Junk

Start: 2014 End: 2027

Linker Junk Sequence probably left over from cloning

HIV cpPu (Trip)

Start: 2034 End: 2211

Central Poly Purine Track

Original Location Description:

2034..2211

Qualifiers:

/gene="cppt"

Human EF1a Promoter

Start: 2218 End: 3402

GS bridge

Start: 4492 End: 4503

F2A optimized

Start: 4504 End: 4575

IRES

Start: 5995 End: 6572

GS bridge

Start: 7527 End: 7538

P2A

Start: 7539 End: 7595

WPRE

Start: 8928 End: 9519

Woodchuck Hepatitis Virus Posttranscriptional Regulatory Element

Feature extends beyond the specified end point

Original Location Description:

3563..4154

Qualifiers:

/gene="WPRE"

HIV nef

Start: 9524 End: 9625

HIV nef

HIV PPT

Start: 9574 End: 9588

PolyPurine Tract

dU3

Start: 9591 End: 9627

1st Half of deleted U3

Insert

Start: 9641 End: 9692

loxP

Start: 9647 End: 9680

dU3

Start: 9695 End: 9713

2nd Half of deleted U3

HIV R

Start: 9714 End: 9810

HIV U5

Start: 9811 End: 9893
HIV U5

SV40 ori

Start: 11987 End: 12110
Original Location Description:
7991..8114
Qualifiers:
/gene="SV40 ori"

PolyA Signal (1 total)

G-T cluster

Start: 570 End: 577
GT Cluster shown to be necessary for Poly-A site activity by Cullen in J Virol. 1989 January;

PolyA Site (1 total)

pA Site

Start: 550 End: 550
Exact base to which Poly-A is added

Primer Binding Site (28 total)

pHAGE-A0050-8050

Start: 158 End: 179 (Complementary)

pHAGE-S501-1000

Start: 401 End: 421

pHAGE-A0550-0050

Start: 667 End: 690 (Complementary)

pHAGE-S1001-1500

Start: 898 End: 919

pHAGE-A1050-0550

Start: 1166 End: 1189 (Complementary)

pHAGE-S1501-2000

Start: 1391 End: 1417

pHAGE-A1550-1050

Start: 1650 End: 1678 (Complementary)

pHAGE-S2001-2500

Start: 1898 End: 1922

pHAGE-A2050-1550

Start: 2084 End: 2112 (Complementary)

pHAGE 5' IRES

Start: 6011 End: 6031

pHAGE 3' CDS 1-1 (Binds IRES)

Start: 6019 End: 6039 (Complementary)

pHAGE 3' CDS 1-2 (Binds IRES)

Start: 6275 End: 6293 (Complementary)

pHAGE 5' CDS 2-1 Start (Binds IRES)

Start: 6275 End: 6293

pHAGE 3' CDS

Start: 8956 End: 8990 (Complementary)

pHAGE-A3550-3050

Start: 9034 End: 9057 (Complementary)

pHAGE-S4001-4500

Start: 9187 End: 9207

oligo #91 sense

Start: 9480 End: 9503

pHAGE-S6001-6500

Start: 9925 End: 9947

pHAGE-A6050-5550

Start: 10198 End: 10221 (Complementary)

pHAGE-S6501-7000

Start: 10398 End: 10422

pHAGE-A6550-6050

Start: 10667 End: 10690 (Complementary)

pHAGE-S7001-7500

Start: 10918 End: 10942

pHAGE-A7050-6550

Start: 11178 End: 11199 (Complementary)

pHAGE-S7501-8000

Start: 11422 End: 11446

pHAGE-A7550-7050

Start: 11689 End: 11710 (Complementary)

pHAGE-S8001-8500

Start: 11925 End: 11944

pHAGE-A8050-7550

Start: 12165 End: 12191 (Complementary)

pHAGE-S1-500

Start: 12430 End: 12458

Promoter Prokaryotic (1 total)

P-Bla

Start: 10096 End: 10130

Beta Lactamase Promoter

Replication Origin (1 total)

ORI

Start: 11217 End: 11750

Bacterial Origin of Replication

Original Location Description:

7221..7754

Qualifiers:

/gene="ORI"

/product="ColE1 origin of replication"

Splicing Signal (3 total)

5' SD 289

Start: 744 End: 745

Major 5' Splice Donor 289

SA 7925

Start: 1911 End: 1912

SA 4459

Start: 2188 End: 2189

Stem Loop (4 total)

SL-1

Start: 697 End: 731
PSI Stem loop 1

SL-2

Start: 736 End: 754
PSI Stem loop 2

SL-3

Start: 766 End: 779
PSI Stem Loop 3

SL-4

Start: 793 End: 806
PSI Stem Loop 4

5' UTR (1 total)

5' UTR

Start: 551 End: 635
HIV 5' UTR

Mutation (3 total)

Fill-in

Start: 833 End: 834
Filled in Cla Site from original HIV genome to disrupt gag polyprotein ORF

RRE Mutation

Start: 1357 End: 1357
This G is a C in the WT HIV-1 genome

EF1a Mutation

Start: 2531 End: 2531

Restriction/Methylation Map

Enzyme	# of cuts	Positions
Acc65I	4	4373 6428 9521 9682
AflII	3	518 2603 9777
ApaLI	5	5401 6464 9139 10247 11493
AscI	1	9641
AvaI	17	296 1928 2269 2371 2557 3072 3189 3848 4217 4492 5026 5134 5578 7125 7588 8299 9895
BamHI	1	5990
BclI	1	7394
BglII	4	474 2790 9557 9733
BsrGI	2	5181 6578
ClaI	1	8381
DraI	7	2062 2725 9574 10341 11033 11052 12493

EagI	9	1145 1149 3405 4726 5444 6662 6838 7880 8012
EcoRI	1	2029
EcoRV	4	36 115 334 7740
EgeI	11	640 3164 4256 4815 5037 5340 6804 7014 7364 7416 7727
FseI	2	1151 2928
FspI	2	7139 10696
HindIII	6	532 1088 1676 3520 6211 9791
KpnI	4	4377 6432 9525 9686
MscI	3	4760 5439 6565
NaeI	6	1149 2926 5448 6666 7017 9444
NcoI	2	3411 7292
NdeI	1	6572
NotI	1	3405
PmlI	3	291 5367 6303
PstI	12	2540 3045 3840 3984 4026 4164 4185 4994 7193 7718 8132 9634
PvuI	2	5708 10550
PvuII	4	436 7107 7890 9552
SacI	7	492 683 3052 4692 5157 5652 9751
SacII	6	2478 2825 6656 6735 8094 9433
SbfI	2	8132 9634
SdaI	2	8132 9634
SfiI	2	3481 12039
SmaI	5	5028 5580 7127 7590 9897
SpeI	1	2213
StuI	4	2595 2638 5058 12085
XcmI	4	3533 5414 7299 7332
XhoI	3	3189 3848 4217
XmaI	5	5026 5578 7125 7588 9895

No cuts: HpaI, MluI, NheI, PaeI, PmeI, Sall, SrfI, Swal, XbaI

Sequence

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cacaaggcta cttccctgat tagcagaact acacaccagg
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agataaggta gaagaggcca ataaaggaga gaacaccagc
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Component Fragments